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APPLICATION OF MULTIVARIATE STATISTICAL ANALYSIS FOR BREEDING STRATEGIES OF SPRING SAFFLOWER (*Carthamus tinctorius* L.)

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ABSTRACT

This study aimed to assess oil yield components and their interrelationships of spring safflower lines and varieties by using different statistical techniques to increase the oil yield in safflower breeding program. Field experiments were conducted at the Transitional Zone Agricultural Research Institute in Eskisehir, Turkey during 2014, 2015 and 2016. Correlation, simple linear regression, stepwise multiple regression, path, principal component and cluster analyze were used to investigate the relationships between oil yield and some components in spring safflower. The results revealed that characters affecting oil yield, which is important to determine selection criteria in plant breeding, vary according to statistical methods. Therefore, to obtain reliable result, it is essential to use multivariate statistical methods for scanning significant characters in studied material. According to the numbers of common characters determined in different statistical analyzes; oil content, seed weight, seed yield and number of head per plant would be important selection criteria for improved oil yield in the breeding material studied. The lines and varieties may be used in hybridization program and their hybrids may yield more transgressive sergeants for these characters for oil yield improvement.

Keywords: Characters associations, Spring safflower, Selection criteria, Statistical analysis

INTRODUCTION

The aim of the breeding researches must be determined carefully in order to achieve success. Generally, safflower breeding program aimed to improve adaptable high-yielding safflower cultivars. Determination of the characters that affect seed and oil yield in safflower is very important in genetic improvement of these attributes (Golparvar, 2011a). Yield is complicated quantitative character that results to the actions and interactions of different characters (Tonk et al., 2011). Especially, breeding programs intended to develop high-yielding genotypes, it is essential to know how and to what extent these factors affect yield. Selection is the essential parts of these researches. The achievement of selection connects with the choice of selection criteria for improving yield (Samonte et al., 1998). Yield components do not only directly affect the yield, but also indirectly by affecting other yield components in negative or positive ways (Bidgoli et al., 2006). Different statistical methods are used for these purposes.

Correlation coefficients used to determine relationships between yield and yield components have importance in selecting breeding materials (Afroz et al., 2004; Laei et al., 2012; Zafarnaderi et al., 2013). Regression analysis is used to establish the existence of linear relationship between the independent variables and dependent variable. While simple linear regression is preferred the presence of single independent variable, multiple regression analysis is used more than one independent variable (Khan and Nagvi, 2012). However, stepwise multiple linear regression is a method that is used to anticipate the value of a quantitative variable concerning its relation with one or some other quantitative variables. This relation is such that it is possible to predict other changes using one variable (Hannachi et al., 2013). In additional, path coefficient analysis focused to determine the direct and indirect influence of each of the component characters on seed yield (Khan et al., 2013). Principal component analysis is multivariate statistical techniques that aim to simplification in complex plant data sets (Slavkovic et al., 2004). Cluster analysis is an agglomerative hierarchical method and classifies variables into groups and to cluster variables reduction their number (Katar, 2013).

Plant height, number of branch per plant, branch height, number of heads per plant, number of seeds per head, head diameter, seed weight and oil content are the most important characters in spring safflower breeding for increasing seed and oil yield (Patil, 1990; Ozturk, 1994; Omidi, 2000; Camas et al., 2005; Topal et al., 2010; Golkar et al., 2011; Golparvar, 2011a; Karimi et al., 2013; Hussain, 2014). These characters are affected from environmental condition and genotypic differences (Camas and Esendal, 2006; Arslan, 2007).

Therefore, the present study was designed to assess yield components and their interrelationships in the present spring safflower material for efficient selection to increase the oil yield in safflower breeding program.

MATERIALS AND METHODS

In this study was carried out at the Transitional Zone Agricultural Research Institute, (39°45''57' N, 30°24'' 5' E) in Eskisehir, Turkey during 2014, 2015 and 2016 under rainfed conditions. Experimental location has typically a steppe climate with temperature differences between day and night and dries in summer, relatively rainy winter. Mean temperatures and total rainfall during experimental period (March – August) in 2014, 2015, 2016 and long-term years' values were 16.6 °C and 207.2 mm, 15.2 °C and 337.3 mm, 16.9 °C and 172.6 mm, 15.1 °C and 166.9 mm, respectively (Table 1). Soils in the research area had clayey and neutral in reaction. It is poor in organic matter and reach in available potassium and phosphorus content.

Table 1. Some climatic data during the 2014, 2015, 2016 growing seasons and long-term values for the experimental area

Mounts	Lowest temperature (°C)	Highest temperature (°C)	Mean temperature (°C)	Relative humidity (%)	Rainfall (mm)
			Long Term (1965-2016)		
March	-9.1	22.1	4.8	62.2	33.6
April	-4.4	26.4	10.1	59.4	44.0
May	0.2	29.7	14.8	56.6	44.4
June	4.1	33.4	18.5	53.5	25.1
July	7.3	35.8	21.4	52.9	10.0
August	6.7	34.7	21.0	52.4	9.8
Mean	0.9	30.5	15.1	56.3	-
Total	-	-	-	-	166.9
Year			**2014**		
March	-5.9	23.0	6.2	69.0	27.1
April	-3.7	26.6	11.3	63.7	23.2
May	6.6	28.5	16.4	63.3	53.8
June	8.9	35.4	19.9	64.1	70.5
July	13.6	34.0	21.6	57.8	20.4
August	15.4	36.4	24.1	58.9	12.2
Mean	5.8	30.6	16.6	62.8	-
Total	-	-	-	-	207.2
Year			**2015**		
March	- 4.7	20.3	5.7	78.6	46.0
April	-4.7	26.3	7.9	68.0	41.3
May	2.5	30.8	15.7	64.7	61.2
June	5.8	28.2	17.2	80.7	125.3
July	10.1	37.2	22.1	63.0	0
August	9.6	33.9	22.7	66.3	63.5
Mean	3.1	29.4	15.2	70.2	-
Total	-	-	-	-	337.3
Year			**2016**		
March	-6.7	23.5	7.5	70.3	44.8
April	-1.9	28.6	12.9	64.4	23.5
May	2.8	29.9	14.2	74.3	55.3
June	4.8	35.3	21.0	62.1	8.7
July	14.1	38.5	22.8	58.2	8.5
August	9.6	36.5	22.8	65.8	31.8
Mean	3.7	32.1	16.9	65.8	
Total	-	-	-	-	172.6

The research plant material comprised of thirty-four spring safflower genotypes included thirty lines and four varieties were sown in plots with 4 rows; plots were 2 m long, with 45 cm between rows and 10 cm between plants within rows in randomized block design with four replications. Seeding was made in the first week of March every year by hand. At seeding, 80 kg ha^{-1} nitrogen (33% ammonium nitrate) and 60 kg ha^{-1} phosphorus (superphosphate) were applied. During growing season weeds were controlled by hand.

The followed data was measured oil yield (OY), seed yield (SY), oil content (OC), plant height (PH), number of branch per plant (NBPP), number of head per plant (NHPP), head diameter (HD) and thousand seed weight (SW). Randomly selected fifteen plants per plot were used to determine yield components. Plants were harvested in August. Data on seed yield were taken from the middle 4 rows of each plot, leaving aside the guard rows on either side of a plot. Oil content of genotype was determined by using soxhlet apparatus. Oil yield was calculated by multiplying oil content and the seed yield of each plot.

Correlation analysis was performed for lines and varieties to analyze the relationships between oil yield and yield characters accurately. Simple linear regression coefficients of oil yield on the different characters studied were analyzed. The stepwise multiple regression analysis was also conducted in for the data provided to test the significance of the independent characters affecting the oil yield. In path analysis; oil yield used as dependent variable, and the other studied characters were use as predictor variables. Beside of these principal component analysis (PCA) was used for understanding the data structure and characters relations. It helps derive a small number of independent linear combinations (principal components) of a set of variables, ways to explore how several variables relate to each other. The platform begins with a standard correlation matrix and gives additional options for correlations and other techniques for looking at several variables. Clustering is a technique of grouping rows together that share similar values across a number of variables.

In this research, Bartlett's test was used to determine the homogeneity of variances for three years. Analysis of variance (ANOVA) was performed and statistically significant differences among the mean values were determined with the least significant difference (LSD) test at the 0.05 level. The data over three years subjected to different statistical analyses, using Jump 5.0.1 and gene stat package programs.

RESULTS AND DISCUSSION

The results of combined variance analysis showed that, genotypes differed significantly at $p < 0.01$ for all characteristics, indicating significant genetic variability present for these characters among genotypes (Table 2). The presence of variability is important for genetic studies and consequently improvement and selection. Beside of this, significant differences among the years and genotype x years was found all investigated characteristics. Significant year effects indicated the presence of variability in the environmental variables for three years. The interaction between years and genotypes indicated that differences between genotypes were affected by the growing season.

Table 2. Combined analysis of variance for studied characters over three years

		Mean squares							
	DF	OY	SY	OC	PH	NBPP	NHPP	HD	SW
Years	2	39445.7**	2914.41**	112.472**	10827.6**	422.451*	882.005**	4.1115**	78.0023*
Genotypes	33	5266.19**	603.925**	55.937**	320.063**	5.85276**	46.8064**	0.16895**	21.6975**
YxG	66	2224**	207.83**	10.1825**	131.274**	0.9552**	8.26812**	0.11969**	8.317**

Means of oil yield varied between 270.7 and 580.3 kg ha^{-1}. The seed yield, oil content, plant height, number of branches, number of head, head diameter and for seed weight between 1002.0 and 1881.0 kg ha^{-1}, 24.7 and 37.4 %, 84.5 and 119.9 cm, 4.6 and 8.5, 12.5 and 23.5, 1.96 and 2.51 cm, 35.2 and 41.1 g, respectively (Table 3). According to results, considerable variation was determined among genotypes provided opportunity for selection.

Correlations coefficients determined among the characteristics are presented in Table 4 positive significant relation were found between oil yield and plant height (r=0.127*), seed weight (r=0.298**), number of branch per plant (r=0.326**), oil content (r=0.344**), number of head per plant (r=0.385**), seed yield (r=0.927**). According to the results any positive increase in such characters will lead to improve in oil yield. These results implied that oil yield would increase if the genotype having higher plant height, number of branch and head per plant, seed weight, oil content and seed yield in maturity would be selected. Some studies reported that significant and positive relationship with oil or seed yield; number of seed per plant, seed weight, head diameter could safely be used breeding programs (Bidgoli et al., 2006; Mozaffari and Asadi, 2006; Topal et al., 2010; Golparvar, 2011b). Beside of this, Behnam et al. (2011) determined positive and significant relation between number of head per plant with oil yield.

Simple regression coefficients (b) of oil yield on the different characters studied were analyzed, together with their Sb values. The significance of the coefficients determined was tested by calculating t values (Table 5). All the b values were found positive and highly significant except the b values for plant height over three seasons. The highest regression coefficient was determined at number of branches per plant. Regression coefficient (b values) for number of branches per plant, oil content, number of head per plant, seed weight, plant height and seed yield were positively significantly correlated with oil yield indicating that increase in these characters would increase the oil yield. Linear regression of number of branch per plant, oil content, seed yield, number of head per plant, seed weight and plant height leads to increase the oil yield by 1.852, 1.319, 0.289, 1.249, 1.472 and 0.116 units, respectively. Presence of highly significant and positive correlation between these characters with oil yield shows that the results of regression analysis are nearly in harmony with correlation results.

Table 3. Mean and least standard deviation (Lsd) values studied characters of thirty-four safflower genotypes over three years

Genotype number	Pedigre	OY (kg ha^{-1})	SY (kg ha^{-1})	OC (%)	PH (cm)	NBPP	NHPP	HD (cm)	SW (g)
1	07.2.1.1.1	341.0	1137.8	29.9	89.5	7.5	20.6	2.16	35.5
2	07.2.1.2.1	285.5	1016.2	28.1	85.5	7.8	21.4	2.19	36.0
3	07.2.1.4.1	336.5	1080.1	31.2	90.8	7.3	19.5	2.11	35.3
4	07.2.2.1.1	414.1	1356.5	30.6	88.8	8.0	22.4	2.03	39.9
5	07.2.2.2.1	318.8	1080.7	29.5	97.0	8.1	21.1	1.97	36.8
6	07.15.2.1	498.5	1666.9	29.9	95.9	7.8	20.9	2.10	39.4
7	07.15.2.2	517.5	1768.5	29.2	96.3	7.4	22.6	2.23	41.1
8	07.15.2.3	439.4	1431.1	30.7	91.2	7.3	21.1	2.29	40.4
9	07.15.3.1	511.6	1654.3	30.8	94.0	8.1	22.5	2.17	40.1
10	08.6.1.1	499.5	1777.7	28.0	90.0	8.1	21.8	2.24	38.3
11	08.6.1.2	407.3	1287.8	31.7	93.6	7.6	19.8	2.13	38.5
12	08.6.7.1	443.6	1478.6	30.0	93.7	6.8	18.6	1.96	38.9
13	08.6.8.1	318.9	1002.0	31.8	98.2	7.4	20.5	2.15	38.4
14	08.6.8.2	315.3	1107.8	28.5	90.9	8.0	19.8	2.26	38.3
15	08.10.1.1	580.3	1881.0	31.1	84.5	8.5	23.1	2.29	41.0
16	08.10.7.1	367.3	1286.5	28.9	96.8	7.2	18.4	2.10	39.1
17	08.13.1.1	456.3	1537.3	30.1	91.3	6.1	17.7	2.44	40.1
18	08.13.2.1	483.2	1486.2	32.8	94.7	6.7	18.2	2.10	37.4
19	08.14.5.1	465.4	1524.6	30.7	96.4	7.2	18.8	2.12	37.7
20	08.14.13.1	450.1	1425.1	31.6	95.7	8.3	22.2	2.18	38.3
21	08.14.13.2	425.6	1393.5	30.4	87.6	8.3	22.1	2.23	38.6
22	08.22.13.1	557.3	1826.6	30.9	94.2	8.5	23.3	2.25	38.2
23	5.70	449.4	1653.1	27.3	91.6	7.2	19.5	2.14	37.4
24	510.1	384.2	1495.2	25.7	92.3	7.5	21.2	2.10	36.3
25	5.37-5.118	359.0	1333.9	26.9	96.1	7.6	20.9	2.30	37.8
26	5.12.1	318.9	1308.3	24.7	92.3	6.5	19.6	2.38	36.6
27	1.07	346.8	1253.9	27.7	88.4	7.2	21.0	2.22	38.1
28	5.154-5.62.1	405.2	1470.0	27.3	85.5	7.4	21.5	2.33	38.2
29	5.154-5.62.2	405.7	1485.9	27.7	86.8	7.5	20.8	2.27	37.6
30	5.75-5.154.2	495.9	1759.1	28.5	91.0	8.4	23.5	2.28	37.0
31	5.122-5.154.2	344.7	1385.6	24.7	93.9	7.6	20.7	2.39	37.4
32	Dinçer	394.3	1384.4	28.5	93.2	5.8	15.1	2.50	36.2
33	Yenice	270.7	1051.7	26.3	119.9	4.6	12.5	2.42	35.2
34	Balcı	549.4	1467.4	37.4	91.2	7.5	18.3	2.51	38.9
Lsd		15.8	190.5	1.4	7.7	0.6	1.6	0.12	1.4

Table 4. The correlation coefficients among the oil yield and studied characters in safflower

	OY	SY	OC	PH	NBPP	NHPP	HD	SW
OY	1	0.927**	0.344**	0.127*	0.326**	0.385**	-0,014^{ns}	0.298**
SY		1	-0.024^{ns}	0.108*	0,330**	0.411**	0.052^{ns}	0.220**
OC			1	-0.061^{ns}	0.046^{ns}	-0.003^{ns}	-0.187^{ns}	0.233**
PH				1	0.398**	0.239**	-0.409**	-0.064^{ns}
NBPP					1	0.790**	-0.452**	0.019^{ns}
NHPP						1	-0.368**	0.131**
HD							1	-0.050^{ns}
SW								1

*and ** = Significant at 5 and 1 % level, respectively, ns = Non-Significant

Table 5. Regression coefficients (b values) of different component traits on oil yield in safflower along with their standard errors, t value and probability value (P), linear regression equations

Characters	Regression values (b)	R^2 adjusted	Standard error of coefficients	t - value	P - value	Linear regression equation
SY	0.289	0.859	0.007	43.13	0.000	OY = 0.67 + 0.289 SY
OC	1.319	0.115	0.206	6.39	0.000	OY = 2.92 + 1.319 OC
PH	0.116	0.086	0.052	2.23	0.026	OY = 30.89 + 0.116 PH
NBPP	1.852	0.103	0.308	6.01	0.000	OY = 27.89 + 1.852 NBP
NHPP	1.249	0.145	0.170	7.26	0.000	OY = 16.30 + 1.249 NHP
SW	1.472	0.081	0.270	5.45	0.000	OY = -14.35 + 1.472 SW

Stepwise regression was used to remove effect of non-effective characteristics in regression model on oil yield. In this analysis, oil yield (Y) as dependent variable and other characters as independent variables were considered. Accepted variables and their relative contributions are given in Table 6. Results of stepwise regression indicated that seed yield and oil content with R square of 99.7%, had justified the maximum of oil yield changes. The relative contributions in the total variation of oil yield were 88.1% for seed yield, 17.9% oil content. The other characters were not included in the model because of their low relative contributions and the best prediction equation was obtained as follows: OY = -40.85 + (0.29 x SY) + (1.39 x OC). A study aimed to determine the best indirect selection criteria for genetic improvement of in spring safflower in terms of oil yield emphasized that seed weight had positive and significant regression coefficient and accounted for 52.0% of total variation exist in oil yield (Karimi et al., 2013).

Table 6. Relative contribution (partial and model R^2), regression coefficient (b), standard error (SE), t-value and probability value (P) in predicting safflower oil yield by the stepwise procedure analysis

Step	Variable entered	Partial R^2	Model R^2	b	SE	t	P-value
1	SY	0.818	0.818	0.292	0.0033	87.33	0.000
2	OC	0.179	0.997	1.397	0.0325	43	0.000
RSquare: 0.9970; RSquare Adj: 0.9968							

Path analysis is effective and convenient method for explaining dependent variable and explains well degrees of direct and indirect effect of yield components on yield (Topal et al., 2004). Table 7 showed that seed yield (0.9311), oil content (0.3653), plant height (0.0076), number of branch per plant (0.0025), number of head per plant (0.0015), head diameter (0.0096), seed weight (0.0086), exerted positive direct effect on oil yield. This result indicated that a slight increase in terms of these characters may directly contribute to oil yield. Meanwhile the highest indirect effect percentage of number of head per plant (97.17) followed by number of branches per plant (91.67), plant height (73.80), seed weight (68.33) was found via seed yield. According to these results; studied characters, except of head diameter, important selection criterion to obtain higher oil yield genotype in spring safflower, Gencer et al. (1987) determined that plant height and seed weight; Patil (1998) seed weight, number of head and branch per plant; Tuncturk and Ciftci (2004) number of head per plant and seed per head; Arslan (2007) head diameter, number of head per plant and number of seed per head had positive direct effect on seed yield. Whereas, Omidi (2000) revealed that number of head per plant had positive direct effect, Mahasi et al. (2006) reported negative direct effect on seed yield. A study conducted rainfed condition, it has been reported that seed weight has a positive direct effect on oil yield (Katar, 2013). It was determined that differences and similarities between the results of the previous studies and this research as a result of the environmental and genotypic differences.

According to principal component analyze results, variable could be grouped in two components and these components account for 65.4% of the total variation of oil yield. The first PC contributed 44.17% of the overall variance of the studied characters, the second PC 20.87% on seed yield, seed weight, number of branch per plant, number of head per plant and oil content (Table 8, Figure 1). These results implied that seed yield, oil content and seed weight showed up to be important characters for oil yield of spring safflower. Studies revealed that correlation between oil yield and seed yield, seed weight, number of branch per plant, number of head per plant and oil content. Mozaffari and Asadi (2006) were observed that for the first principal component plant height, head diameter, oil content had positive correlation between them. A study was conducted to 30 spring safflower genotype by Ahmadzadeh (2013) found that three principal components with eigenvalue more than one which contributed 72.92 present of the total variability. Determination the effective plant characters will play important role to increase breeding programs in which success in it highly depends on straightness and effectiveness of yield components (Katar, 2013).

Table 7. Path coefficient (direct and indirect effects) analysis of studied characters on oil yield in safflower over the three years

Path coefficient Percentage (%)	SY	OC	PH	NBPP	NHPP	HD	SW
SY	**0.9311**	-0.0086	0.0008	0.0008	0.0006	0.0005	0.0019
	98.59	0.91	0.08	0.08	0.06	0.05	0.20
OC	-0.020	**0.3653**	0.0050	0.0001	0.0001	-0.0018	0.0020
	5.62	**93.26**	0.12	0.02	0.01	0.45	0.51
PH	0.1004	0.0222	**0.0076**	0.0010	0.0004	-0.0039	-0.0006
	73.80	16.33	**5.57**	0.73	0.26	2.88	0.41
NBPP	0.3069	0.0167	0.0030	**0.0025**	0.0012	-0.0043	0.0002
	91.67	4.97	0.90	**0.74**	0.36	1.29	0.04
NHPP	0.3828	-0.0012	0.0018	0.0020	**0.0015**	-0.0035	0.0011
	97.17	0.29	0.46	0.51	**0.39**	0.89	0.28
HD	0.0488	-0.0682	-0.0031	-0.0011	-0.0006	**0.0096**	0.0004
	37.05	51.72	2.35	0.85	0.42	**7.27**	0.32
SW	0.2046	0.0850	-0.0005	0.0001	0.0002	0.0005	**0.0086**
	68.33	28.37	0.16	0.02	0.06	0.16	**2.88**

Bold figures denotes direct effects while regular figures denote indirect effects

Table 8. Principal components of spring safflower genotype

Traits	PC1	PC2
Percent of variance	44.17	20.87
Cumulative variance	44.17	65.04
Eigenvectors		
Seed yield	0.388	0.357
Oil content	0.223	0.222
Number of branch per plant	0.420	-0.392
Number of head per plant	0.427	-0.377
Plant height	-0.321	0.292
Head diameter	-0.106	0.442
Seed weight	0.374	0.267

Cluster I includes plant height and seed yield while head diameter and number of branches per plant belonged to Cluster II (Figure 2). Cluster III consisted of seed weight, number of head per plant, oil yield and content. These results revealed that, seed weight, number of head per plant and oil content could be regarded as important characters for high oil yielding genotypes in spring safflower breeding programs. A research was conducted to 5 spring safflower genotype by Katar (2013) showed seed weight, oil content and seed yield per plant as important characters to obtained high oil yielding safflower genotypes in cluster analysis.

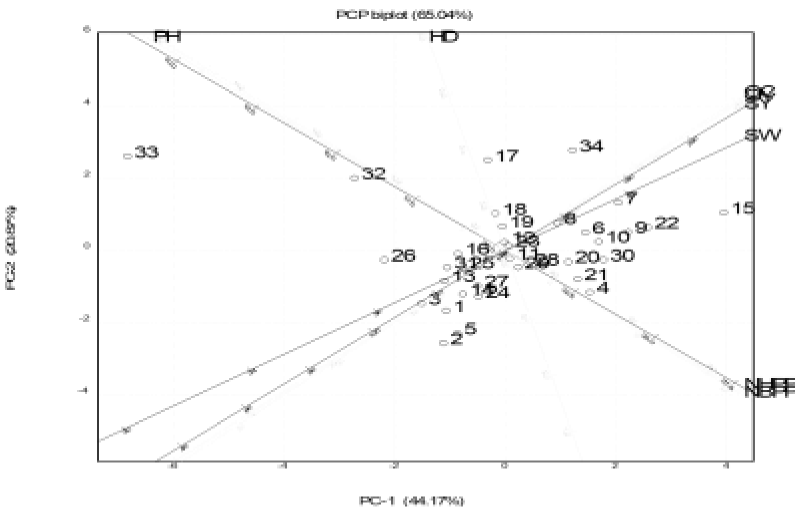

Figure 1. Scatter diagram of spring safflower genotype and studied characters

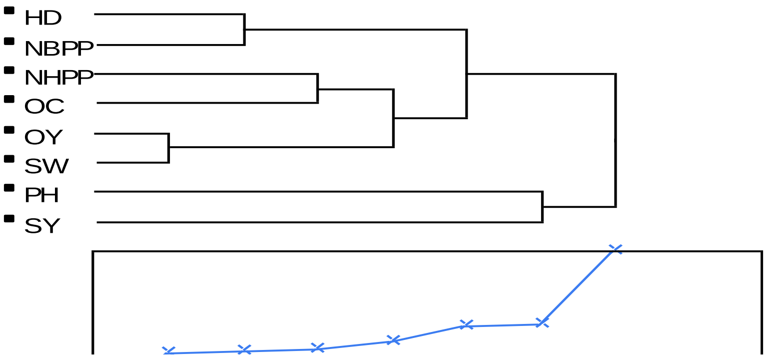

Figure 2. Cluster of characters having different distance levels

According to the results of statistical analyses essential variables effective on oil yield in spring safflower in this study are given Table 9. According to the multiple statistical procedures used in this research revealed that the selection based on oil content, seed weight, seed yield and number of head per plant this material would probably the most useful characters for increasing oil yield in spring safflower. The result of present study could be exploited in planning and execution of future breeding program in spring safflower.

Table 9. Results of statistical analyses indicating effective characters on oil yield in spring safflower

Characters	1	2	3	4	5	6
SY	X	X	X	X	X	
OC	X	X	X	X	X	X
PH	X	X		X		
NBPP	X	X		X		
NHPP	X	X		X		X
HD						
SW	X	X		X	X	X

1: Correlation analysis 2: Multiple linear regression analysis 3: Stepwise analysis, 4: Path analysis 5: Principal component analysis 6: Cluster analysis.

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